Australian Government
Department of Defence
Defence Science and
Technology Organisation

Generation of a Gene Expression Cassette to Enable the Rapid Production of DNA Vaccines

David Proll and Penelope J. Gauci

CBRN Defence Centre
Platforms Sciences Laboratory

DSTO-TR-1597

ABSTRACT

Recombinant DNA techniques have been used to engineer a DNA vaccine from a gene derived from Burkholderia pseudomallei. This vaccine was further manipulated to generate a gene expression cassette that can be used to readily generate new DNA vaccines, utilising genetic material from any source. The DNA vaccine was transfected into mammalian cells, grown in vitro, in order to assess the ability of the DNA to provide the cell with the necessary information for it to synthesise the protein encoded within the vaccine. Western blot analysis of these cell extracts resulted in the detection of a protein with a molecular mass predicted for the protein encoded by the DNA vaccine. This evidence suggests that transient transfection of a mammalian cell with the engineered plasmid DNA construct, resulting in the translation of the encoded protein has been successful. This in vitro process effectively mimics that which occurs when the DNA plasmids are used to vaccinate higher mammals.

Published by

DSTO Platforms Sciences Laboratory
506 Lorimer St
Fishermans Bend, Victoria 3207 Australia

Telephone: (03) 9626 7000
Fax: (03) 9626 7999

AR-013-137
July 2004

APPROVED FOR PUBLIC RELEASE

Generation of a Gene Expression Cassette to Enable the Rapid Production of DNA Vaccines

Executive Summary

ADF personnel are at risk of being exposed to biological warfare (BW) agents deliberately released by an enemy, and to agents occurring naturally in environments where they are deployed. Current BW protective ensembles restrict the soldier's capability when worn over extended periods of time, and therefore are not suitable for constant use under normal operating conditions. This creates the potential for exposure to BW agents during the period between release and detection. Vaccination against such potential agents can provide protection from infection.

Traditional vaccines have been used effectively for the last fifty years, in some cases eliminating diseases such as smallpox and polio. More recently, scientific advances have seen the use of recombinant DNA techniques to produce DNA vaccines. These DNA vaccines act by providing the host with a set of instructions for the synthesis of the encoded antigen. The host cell utilises this information to synthesise the protein that interacts with the host immune system to generate the immune response.

DNA vaccines have the advantage over traditional vaccines in that they may be produced with relative ease and speed. In addition, they have few side effects associated with them and therefore may be more suitable for military personnel who require immunisation with multiple vaccines over a very short time span.

This report outlines the method used to produce a DNA vaccine using a gene derived from *Burkholderia pseudomallei,* a potential BW agent. The gene of interest was amplified from the genomic DNA using the Polymerase Chain Reaction (PCR) and then cloned into a vector to produce the DNA vaccine. The DNA vaccine was assessed for its ability to direct protein synthesis by introducing it into a mammalian cell line grown *in vitro.* Subsequent analysis of the cells revealed the presence of a protein with the molecular weight predicted for the protein that the vaccine was engineered to produce. This demonstrated that the appropriate transcription and translation signals required to produce the protein encoded by the DNA vaccine construct are present. The vaccine was designed and constructed in such a way that the gene of interest may be removed easily and replaced with genetic material from any source to create new vaccines. Such a generic gene expression cassette will reduce the time and effort required to produce new DNA vaccines.

Authors

David Proll
Chemical, Biological, Radiological and Nuclear Defence Centre

David Proll graduated from Monash University in 1993 with a B.Sc(Hons) and went on to complete his PhD in the department of Microbiology. His PhD studies focused on the replication of positive strand RNA viruses. After graduating from university he worked at the Eijkman Institute of Molecular Biology in Jakarta, Indonesia. Here, he investigated the application and development of DNA based vaccines against the parasite that causes Malaria. Upon returning to Australia in 2000, he was recruited by DSTO to initiate a research program investigating DNA vaccines for defence applications.

Penelope J. Gauci
Chemical, Biological, Radiological and Nuclear Defence Centre

Penny Gauci graduated from the University of Melbourne with a B Sc in 1992. She joined the Department of Applied Biology and Biotechnology, RMIT University as a Technical Assistant in 1992, before being promoted to Technical Officer in 1993. Her work at RMIT included production and purification of polyclonal and monoclonal antibodies, antigen purification, chromatography, vaccine production and animal trials for vaccine efficacy testing. She joined AMRL in 1998 and worked predominantly on the production of antibodies for use in ion channel switch (ICS) biosensors. She joined the DNA vaccines program in 2001, and began work on the production of DNA vaccines for Burkholderia pseudomallei.

Contents

1. Introduction

Vaccines are effective at protecting not only individuals, but also communities. In the last fifty years, global immunisation programs have developed such strong "herd immunity" that some diseases, *eg* smallpox and polio, have been effectively eliminated. As scientific advances have been made, the production of vaccines has shifted from traditional means, such as the isolation of attenuated strains (6, 8, 12, 27, 28, 30, 34, 36), to methods that utilise recombinant DNA techniques. These new vaccines may be subunit type protein vaccines (5, 9, 13, 14, 18, 19, 25, 33) or, more recently, vaccines that are composed entirely of DNA (7, 17, 24, 26). These DNA vaccines act by providing the host with a set of instructions for the synthesis of the encoded antigen. The host cell then utilises this information to synthesise the protein that interacts with the host immune system to generate an immune response (32, 35).

Advances in the ability to precisely cut and join together pieces of DNA, have enabled a vast array of genes from one species to be expressed in a heterologous system (2, 11, 15, 16, 20). Here we report the use of these techniques to engineer a DNA vaccine encoding a gene derived from *Burkholderia pseudomallei*. Furthermore, the vaccine was designed as a gene expression cassette that can be readily used to generate new DNA vaccines using any source of genetic material.

2. Materials and Methods

2.1 Bacterial Strains and Plasmids

The plasmid pNB06 was purchased from Ifor Beacham (Griffith University, Queensland, Australia). This plasmid contained a portion of the Bpa A gene sequence from *Burkholderia pseudomallei* (nt 1-4470 plus upstream sequence, approximately 5.5kb in total) cloned into the pBluescript II SK$^+$ commercial vector. The pGem T-easy vector was purchased from Promega and the pcDNA3.1 V5-His vector was purchased from Invitrogen. The general laboratory strains of *E. coli* used in this study included Able K and DH5α (Stratagene). Bacterial cultures were grown overnight in LB broth (Sigma) at 37°C with vigorous shaking. Where appropriate, cultures were supplemented with 50 μg / ml ampicillin.

2.2 Polymerase Chain Reaction (PCR)

The PCR mixture contained 1 ng plasmid DNA (pNB06) template, 40 pmols each of the sense (5' AGCCGCCATGGCCCTCGAGAGTCCCTCTAGAGCGCCGAC GGGTAGTCAG 3', termed Ag1 start) and antisense (5' CCATCTGGCCCATGCTGC 3', termed Ag1 end) oligonucleotide primers (synthesised by GeneWorks), 2 mM each dNTP (Promega), 5 U taq DNA polymerase (Promega), 1 x PCR buffer (10 mM Tris-HCl [pH 9.0], 50 mM KCl, 0.1% Triton X-100) (Promega) in a total volume of 50μl. Thermal cycling parameters, performed using a Peltier Thermal Cycler-200 (MJ Research), were as follows: 94°C for 5 minutes, 50°C for 30 seconds, 72°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 55°C for 15 seconds, 72°C for 90 seconds. A final cycle of 94°C for 30 seconds, 55°C for 15 seconds and an extension of 72°C for 5 minutes concluded the thermal cycling. PCR generated DNA was

visualised by UV light after electrophoresis through a 1% w/v agarose gel in TAE (40 mM Tris-Acetate, 1mM EDTA) containing 0.5 μg/ml ethidium bromide.

2.3 Recombinant DNA techniques

DNA ligations, restriction endonuclease digestion and agarose gel electrophoresis were performed according to standard techniques (31). Enzymes were purchased from Promega. DNA was purified from agarose gels using the QIAEX II gel extraction kit (QIAGEN) as per manufacturer's recommendations. Following ligation of DNA fragments, plasmid DNA was introduced into *E. coli* cells, made competent by calcium chloride treatment, using the heat shock method (31).

2.4 Plasmid DNA preparation

Small-scale plasmid DNA preparations were made from 1.5 mls of a bacterial culture that had been grown overnight in LB broth (Sigma) supplemented with 50 μg /ml ampicillin. Plasmid DNA was extracted from the bacterial cells using the Promega Wizard plasmid purification kit as per manufacturer's recommendations.

2.5 Transfection of 293S cells

Six well plates were seeded with $1x10^6$ 293S cells (Invitrogen) per well in 2mL of growth medium (5% Bovine Calf Serum (ThermoTrace) / DMEM (ThermoTrace) / 5 μg /ml penicillin-streptomycin) and incubated overnight at 37°C, 5% CO_2. Two μg of plasmid DNA in 250 μl SF-DMEM (ThermoTrace) per well to be transfected, was added to 10 μl of Lipofectamine 2000 (Invitrogen) in 250 μl SF-DMEM that had been incubated for 5 minutes at room temperature. The mixture was then incubated for 20 minutes at room temperature. The cells were washed with 1ml SF-DMEM, and 2 ml of SF-DMEM placed over the cell monolayer. The plasmid DNA- Lipofectamine 2000 mixture was added to the appropriate well containing the cell monolayer in 2 ml of SF-DMEM and incubated for 6 hrs at 37°C, 5% CO_2. The 2 ml of SF-DMEM was removed and replaced with 2 ml of the growth medium. The cells were then incubated for a further 24 hrs at 37°C, 5% CO_2.

The cells were harvested by the addition of 1ml of PBS, scraped off and transferred into a microfuge tube. The cells were washed twice by the addition of 500 μl of PBS followed by centrifugation at 200g for 5 minutes to pellet the cells. The cell pellet was resuspended in 100 μl of lysis buffer and incubated on ice for 15 minutes. The solution was then passed through a 22-gauge needle 10 times to shear the DNA.

2.6 Polyacrylamide gel electrophoresis

An equal volume of 2x Laemmli sample buffer (Sigma) (12.2 mM Tris-HCl pH 6.8, 20% Glycerol v/v, 4% SDS w/v, 10% β-mercaptoenthanol v/v, 0.004% Bromophenol blue w/v) was added to the cells harvested above. Cell extracts were fully denatured by heating to 100°C for 5 minutes. The samples were electrophoretically separated through a 12.5% homogeneous SDS polyacrylamide PhastGel (Pharmacia Biotech) as per manufacturer's instructions. Low range, pre-stained molecular weight standards (BioRad) were also loaded onto the polyacrylamide gel to enable molecular weight comparisons. Following electrophoresis, gels were either stained with Coomassie Blue (Sigma) or

electrophoretically transferred to a nitrocellulose membrane (BioRad) using the PhastSystem (Pharmacia Biotech) as per manufacturer's instructions.

2.7 Western blot analysis

Western blot analysis was performed using standard techniques. In brief, following blocking of the membrane in 10 ml of Blocking buffer (5% milk powder in PBS -Tween 20 [Sigma]) for 1 hr at room temperature the membrane was washed twice in 20 ml of PBS-Tween 20. The membrane was then incubated in 5 ml of blocking buffer containing Anti-V5 antibody (Invitrogen) at a dilution of 1:5000, for 2 hrs at room temperature. The membrane was washed twice in 20 ml of PBS-Tween 20 and incubated in 15 ml of blocking buffer containing goat anti-mouse IgG alkaline phosphatase conjugate (Sigma) at a dilution of 1:30000 for 1hr at room temperature. Following washing of the membrane twice in 20 ml of PBS-Tween 20, proteins reactive to the Anti-V5 antibody were then visualised by developing the blot using Sigma Fast BCIP /NBT tablets (5-bromo-4-chloro-3-indolyl phosphate / Nitro blue tetrazolium) as per manufacturer's instructions. The reaction was stopped, by washing the membrane several times in distilled water.

3. Results and Discussion

3.1 PCR Amplification and cloning

The transcription and translation signals that are recognised by prokaryotic (eg bacteria) and eukaryotic (eg mammalian) cells are quite different (1, 3, 4, 10, 23, 29). For example, it has been experimentally determined that for optimal protein translation initiation in eukaryotes, certain nucleotide residues, known as the Kozak consensus sequence, must surround the translation start codon (21, 22). Hence when expressing a gene sequence of bacterial origin within a eukaryotic cell, it is necessary to consider the nucleotides that surround the translation initiation codon. A start codon surrounded by the Kozak consensus sequence was engineered to give optimal expression of the gene in the mammalian host. This was achieved by designing a synthetic oligo-nucleotide primer that contained an ATG translation initiation codon, flanked by the appropriate nucleotides of a Kozak consensus sequence. Also in the primer were a Xho I restriction endonuclease recognition site along with a Xba I restriction site that was in frame with the ATG start codon. Finally, the primer contained 18 nucleotide residues that were homologous to a region of the *Burkholderia pseudomallei* Bpa A gene (figure 1A). This sense oligo-nucleotide primer was designated Ag1 start. The second synthetic oligo-nucleotide primer designed, was designated Ag1 end, and contained 19 nucleotide residues homologous to the anti-sense strand of a region approximately 1100 bp downstream of the sense oligo-nucleotide primer within the BpaA gene.

The polymerase chain reaction was used to amplify a region of the Bpa A gene (nt 152-1227) using the two oligo-nucleotide primers Ag1 start and Ag1 end, and a plasmid DNA template (pNB06) that consisted of a portion of the Bpa A gene cloned into the general purpose vector pBluescript II SK plasmid (Stratagene). Using a standard PCR mutagenesis technique, the amplification of the DNA fragment (figure 1) enabled some specific modification of the DNA sequence to occur. The

procedure enabled a translation initiation codon, a Kozak consensus sequence along with nucleotide sequences coding for restriction endonuclease recognition sequences, to be introduced into the amplified DNA fragment (figure 1A). Following amplification, the DNA fragment was visualised after electrophoresis through an agarose gel (figure 1B). By comparison to DNA fragments of known sizes (figure 1B, lane 1) the length of the amplified fragment is approximately 1100 base pairs (figure 1B, lane2). This corresponds to the calculated size of 1104 base pairs, consisting of 1075 bp of Bpa A gene sequence plus the addition of 29 bp sequence consisting of a Kozak consensus sequence, translation initiation codon and restriction endonuclease recognition sequences.

The PCR generated DNA fragment was cloned into the commercial pGEM-T easy (Promega) cloning vector to generate the plasmid pBps010 (figure 2). This was an intermediate cloning step used to clone the PCR amplified fragment, and more readily permit downstream cloning procedures to be performed. Subsequently an Eco RI digest of this plasmid excised a fragment that consisted predominantly of the cloned PCR fragment. This Eco RI fragment was then cloned into the plasmid vector pcDNA 3.1 V5-His (Invitrogen), which had been digested with Eco RI (figure 2). This generated the plasmid pBps110, for use as a DNA vaccine.

The Eco RI restriction site within pcDNA 3.1 V5-His is flanked by the strong mammalian promoter CMV, and the Bovine Growth Hormone (BGH) transcription termination / polyadenylation signal. Thus the insertion of the Eco RI fragment from pBps010 provides the complete requirement for protein synthesis of the Bpa A gene product within a mammalian cell. That is, the Bpa A gene sequence is preceded by a promoter to drive transcription and is followed by a transcription termination / polyadenylation signal. This will permit a messenger RNA molecule to be synthesised in the nucleus of the cell into which the plasmid DNA is introduced, and for it to be appropriately terminated and polyadenylated. The polyadenylation process is essential for the molecule to be exported out of the nucleus and into the cytoplasm of the cell, where it will provide the necessary instructions to the translational apparatus of the cell and allow synthesis of the encoded protein derived from the *B. pseudomallei* Bpa A gene.

A final point to note with this plasmid, are the nucleotide sequences that occur after the 3′ end of the Bpa A coding region. Following this sequence are the nucleotide residues coding for the amino acids that form a V5 epitope and 6 x His tag followed by a translation termination codon. These sequences are contained within the pcDNA 3.1 V5-His vector and the introduction of the Eco RI fragment was designed in such a manner that the V5 and 6 x His would be in frame with the sequence from the Bpa A gene. Consequently upon translation of the entire reading frame, a protein would be synthesised that contained the amino acid sequence of the portion of the Bpa A protein and also contained a carboxy-terminal V5 epitope and a 6 x His tag. The V5 epitope could then be used to detect the presence of the synthesised protein using an anti-V5 antibody and the 6 x His sequence could be used either to detect the protein in a similar fashion or as a means to purify the protein should this be required.

3.2 Gene expression in vitro

To assess whether the constructed DNA vaccine contained the appropriate signals for directing the cells protein synthesis machinery, the DNA vaccine was introduced into a mammalian cell line, 293S, derived from human kidney cells. The

293S cells were grown *in vitro* and transfected using a cationic lipid complex. If the appropriate transcription and translation signals are present, the plasmid DNA should permit transcription of the encoded gene to produce an mRNA template. Subsequent translation of this template by the 293S cells' ribosomal complexes should result in synthesis of the protein encoded by the DNA vaccine construct. This process mimics that which should occur following the introduction of the DNA vaccine into an animal host. Following the introduction of the DNA into the cells, they were permitted to continue protein synthesis for a further 24 - 48 hours to allow the specific protein to accumulate to levels that could be detected through western blot analysis.

The cells were harvested, lysed and the cellular protein contents separated according to their molecular weight by electrophoresis through a 12.5 % polyacrylamide gel. Proteins were then visualised by staining with coomassie blue (figure 3A). Because of the vast multitude of proteins contained within a cell, it is difficult to distinguish between untransfected 293 cells (lane 3), cells transfected with a positive control plasmid that encodes the Lac Z gene (lane 2) or cells transfected with the experimental plasmid DNA, pBps110 (lane 1).

Therefore to enable the detection of specific proteins within the cell extracts, the proteins from an equivalent gel to that represented in figure 3A were electrophoretically transferred to a solid nitrocellulose membrane. The membrane was then blocked and probed with an antibody directed against the V5 epitope (figure 3B). No proteins contained within a cell extract derived from the 293 cells only (lane 3) reacted with the antibody. In contrast, in lane 2 an immuno reactive protein with a molecular mass in excess of 106 kDa was observed. This lane contained a cell extract derived from 293 cells that had been transfected with the positive control plasmid pcDNA3.1 Lac Z. A protein of this molecular mass would correspond to the lac Z gene product, which has a molecular mass of 121kDa. This indicates that the transfection procedure was successful and the DNA plasmid was able to provide the cell with the necessary information to enable it to synthesise the encoded protein. Likewise in lane 1, an immuno reactive protein is present. This protein has an estimated molecular mass of 40kDa. Contained in this lane are the proteins extracted from 293 cells that had been transfected with the plasmid pBps110. This plasmid was the engineered DNA vaccine that encoded a gene derived from a portion of the Bpa A gene of *Burkholderia pseudomallei* and the molecular weight observed for the detected protein correlated with the expected molecular weight (40kDa) for the encoded protein.

The detection of a protein of the expected size that is reactive against the anti V5 antibody is an indication that the engineering of the DNA vaccine, including the introduction of the ATG start codon and the Kozak consensus sequence, has produced a piece of circular plasmid DNA that is capable of permitting the encoded protein to be synthesised within the transfected mammalian cells. It also provides an indication that the entire open reading frame is undisturbed. As the detection of the protein is reliant upon the protein containing a V5 epitope, which had been engineered into the 3′ end of the gene construct, the motif will therefore only be contained within the protein if the entire open reading frame is translated. This information provides evidence that the engineered plasmid DNA construct should be functional, in terms of permitting protein synthesis when used as a DNA vaccine.

3.3 Generation of a multi use gene expression cassette

An Xba I restriction endonuclease digestion of the plasmid pBps110 (figure 2) generates two fragments. One fragment is approximately 5500 nucleotides in length, the other 1100 nucleotides. The shorter of the two fragments will contain the *B. pseudomallei* Bpa A gene sequence, while the larger fragment will contain sequences that permit the plasmid to replicate within bacterial cells, along with eukaryotic signals for transcription and translation. Separation and isolation of this larger fragment allows the insertion of new DNA fragments to generate new DNA vaccine plasmids. Re-circularisation of this larger fragment, through enzymatic ligation generated the gene expression cassette, pcDNA 3.1 Start (figure 2). Digestion of this plasmid with the restriction endonuclease Xba I, will then permit the insertion of any DNA fragment containing compatible termini. These fragments will be flanked by the appropriate transcription and translation signals required for protein synthesis in eukaryotic cells. Thus, any number of new DNA vaccines can be generated through the simple insertion of gene sequences into this Xba I digested plasmid (figure 2). Using rapid molecular biology DNA amplification techniques, such as PCR, DNA gene sequences can be amplified from genomic DNA (or RNA) while at the same time incorporating Xba I restriction endonuclease recognition sequences to aid in the construction of new DNA vaccines.

In summary, this report has described the engineering and production of:

- a candidate DNA vaccine for B. pseudomallei that was demonstrated to be functional in mammalian cells.
- the plasmid pcDNA 3.1 Start, a gene expression cassette.

This plasmid will be a valuable tool for the generation of DNA vaccines, against both bacteria and viruses, that can subsequently be assessed for their effectiveness as vaccines against a range of potential biological warfare agents.

4. References

1. **Beyersmann, D.** 2000. Regulation of mammalian gene expression. Exs **89**:11-28.
2. **Bienkowska-Szewczyk, K., and B. Szewczyk.** 1999. Expression of genes coding for animal virus glycoproteins in heterologous systems. Acta Biochim Pol **46**:325-39.
3. **Boelens, R., and C. O. Gualerzi.** 2002. Structure and function of bacterial initiation factors. Curr Protein Pept Sci **3**:107-19.
4. **Brock, S., K. Szkaradkiewicz, and M. Sprinzl.** 1998. Initiation factors of protein biosynthesis in bacteria and their structural relationship to elongation and termination factors. Mol Microbiol **29**:409-17.
5. **Chakravarti, D. N., M. J. Fiske, L. D. Fletcher, and R. J. Zagursky.** 2000. Mining genomes and mapping proteomes: identification and characterization of protein subunit vaccines. Dev Biol (Basel) **103**:81-90.

6. **Curtiss, R., 3rd.** 2002. Bacterial infectious disease control by vaccine development. J Clin Invest **110**:1061-6.
7. **Davis, H. L., and M. J. McCluskie.** 1999. DNA vaccines for viral diseases. Microbes Infect **1**:7-21.
8. **Dietzschold, B., and M. J. Schnell.** 2002. New approaches to the development of live attenuated rabies vaccines. Hybrid Hybridomics **21**:129-34.
9. **Engler, O. B., W. J. Dai, A. Sette, I. P. Hunziker, J. Reichen, W. J. Pichler, and A. Cerny.** 2001. Peptide vaccines against hepatitis B virus: from animal model to human studies. Mol Immunol **38**:457-65.
10. **Ganoza, M. C., M. C. Kiel, and H. Aoki.** 2002. Evolutionary conservation of reactions in translation. Microbiol Mol Biol Rev **66**:460-85, table of contents.
11. **Geisse, S., H. Gram, B. Kleuser, and H. P. Kocher.** 1996. Eukaryotic expression systems: a comparison. Protein Expr Purif **8**:271-82.
12. **Gershon, A. A.** 2001. The current status of live attenuated varicella vaccine. Arch Virol Suppl:1-6.
13. **Good, M. F., H. Xu, and M. Batzloff.** 2002. Adapting immunity with subunit vaccines: case studies with group A Streptococcus and malaria. Int J Parasitol **32**:575-80.
14. **Green, B. A., and S. M. Baker.** 2002. Recent advances and novel strategies in vaccine development. Curr Opin Microbiol **5**:483-8.
15. **Griffiths, C. M., and M. J. Page.** 1997. Production of heterologous proteins using the baculovirus/insect expression system. Methods Mol Biol **75**:427-40.
16. **Gross, G., and H. Hauser.** 1995. Heterologous expression as a tool for gene identification and analysis. J Biotechnol **41**:91-110.
17. **Gurunathan, S., D. M. Klinman, and R. A. Seder.** 2000. DNA vaccines: immunology, application, and optimization*. Annu Rev Immunol **18**:927-74.
18. **Hansson, M., P. A. Nygren, and S. Stahl.** 2000. Design and production of recombinant subunit vaccines. Biotechnol Appl Biochem **32 (Pt 2)**:95-107.
19. **Jenkins, M. C.** 2001. Advances and prospects for subunit vaccines against protozoa of veterinary importance. Vet Parasitol **101**:291-310.
20. **Kost, T. A., and J. P. Condreay.** 1999. Recombinant baculoviruses as expression vectors for insect and mammalian cells. Curr Opin Biotechnol **10**:428-33.
21. **Kozak, M.** 1987. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res **15**:8125-48.
22. **Kozak, M.** 1987. At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. J Mol Biol **196**:947-50.
23. **Kozak, M.** 1992. Regulation of translation in eukaryotic systems. Annu Rev Cell Biol **8**:197-225.
24. **Lai, W. C., and M. Bennett.** 1998. DNA vaccines. Crit Rev Immunol **18**:p449-84.
25. **Liljeqvist, S., and S. Stahl.** 1999. Production of recombinant subunit vaccines: protein immunogens, live delivery systems and nucleic acid vaccines. J Biotechnol **73**:1-33.
26. **Liu, M. A.** 2003. DNA vaccines: a review. J Intern Med **253**:402-10.
27. **Mills, J., R. Desrosiers, E. Rud, and N. Almond.** 2000. Live attenuated HIV vaccines: a proposal for further research and development. AIDS Res Hum Retroviruses **16**:1453-61.
28. **Murphy, B. R., and P. L. Collins.** 2002. Live-attenuated virus vaccines for respiratory syncytial and parainfluenza viruses: applications of reverse genetics. J Clin Invest **110**:21-7.
29. **Ramakrishnan, V.** 2002. Ribosome structure and the mechanism of translation. Cell **108**:557-72.

30. **Ryan, E. T., and S. B. Calderwood.** 2000. Cholera vaccines. Clin Infect Dis **31**:561-5.
31. **Sambrook, J. F., EF. Fritsch and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Sprong Harbor Laborator Press, Cold Sprong Harbor, New York.
32. **Tang, D. C., M. De Vit, and S. A. Johnston.** 1992. Genetic immunization is a simple method for eliciting an immune response. Nature **356**:152-4.
33. **Tsuji, M., and F. Zavala.** 2001. Peptide-based subunit vaccines against pre-erythrocytic stages of malaria parasites. Mol Immunol **38**:433-42.
34. **Wareing, M. D., and G. A. Tannock.** 2001. Live attenuated vaccines against influenza; an historical review. Vaccine **19**:3320-30.
35. **Wolff, J. A., R. W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani, and P. L. Felgner.** 1990. Direct gene transfer into mouse muscle in vivo. Science **247**:1465-8.
36. **Zangwill, K. M.** 2003. Cold-adapted, live attenuated intranasal influenza virus vaccine. Pediatr Infect Dis J **22**:273-4.

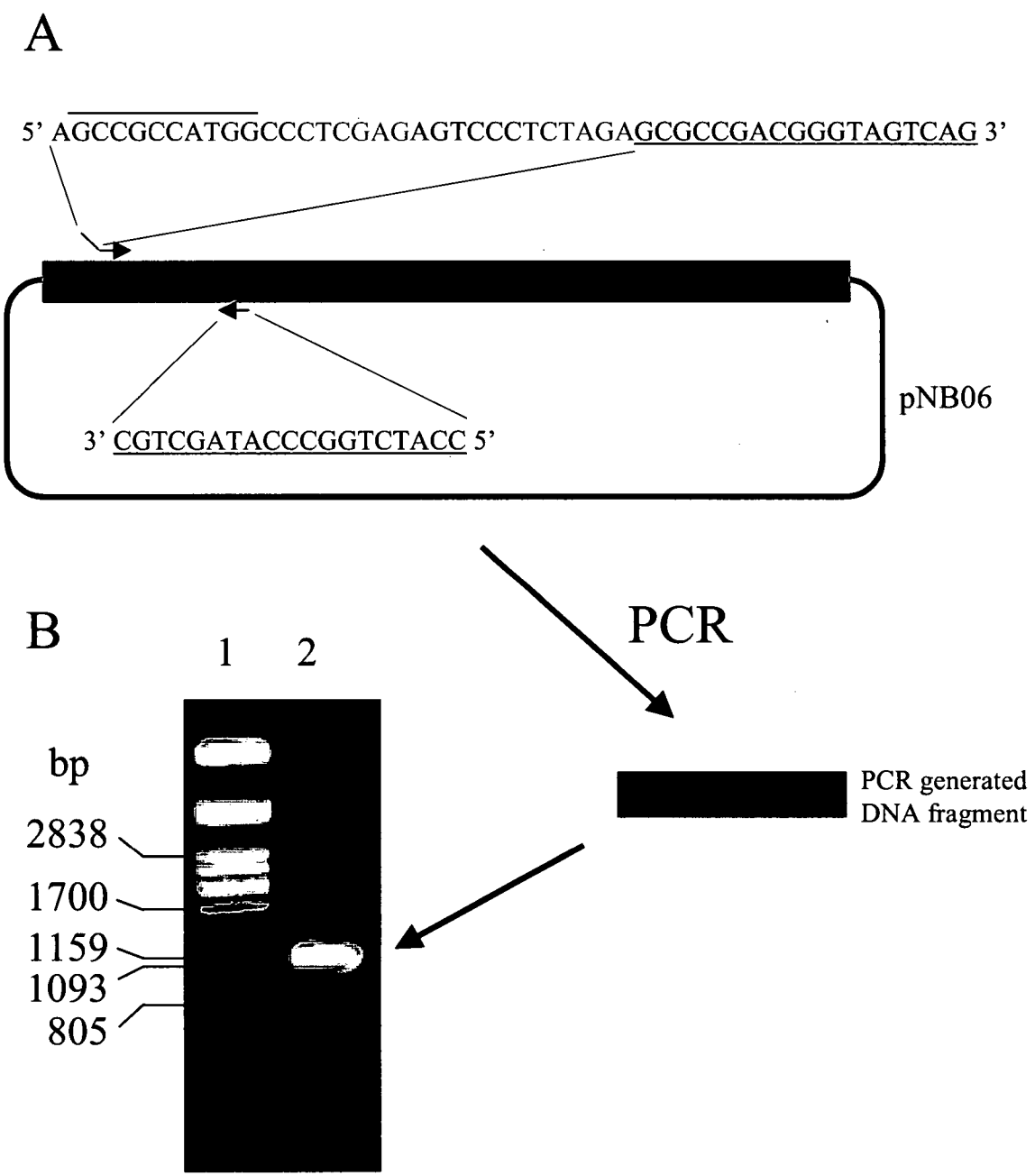

Figure 1: Schematic representation of the amplification and engineering of the expression cassette. The Polymerase Chain Reaction (PCR) was used to amplify the specific *Burkholderia pseudomallei* (orange box) sequence of interest, with the plasmid pNB06 as a template (A). The nucleotide sequence of the reverse and forward primer is shown. Within the forward primer, the translation start codon is in red, an Xho I (green) and Xba I (blue) recognition sequence is highlighted, while the *Burkholderia pseudomallei* specific sequence is underlined and a line is above the Kozak concensus sequence. The amplified DNA is visualized following electrophoresis through a 1% agarose gel and staining with ethidium bromide (B). Lane 1 contains λ DNA digested with Pst I as size markers and indicated in base pairs (bp). Lane 2 contains the contents from a single PCR reaction.

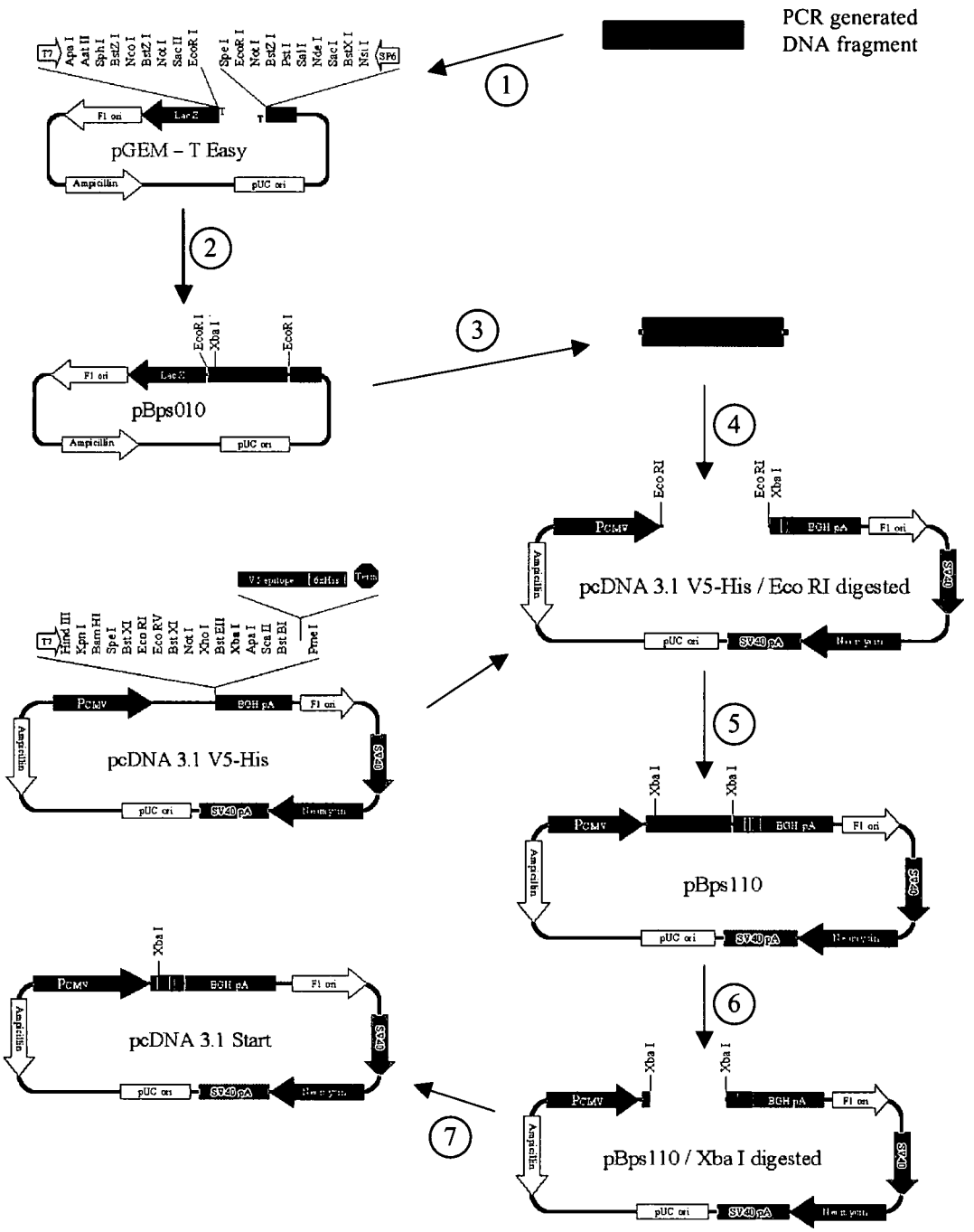

Figure 2 : Schematic representation of the construction of the DNA vaccine pBps110.
The Polymerase Chain Reaction (PCR) was used to amplify a the specific gene of interest. The amplified DNA was cloned into the pGEM-T Easy vector (1) to generate the plasmid pBps010 (2). The Eco RI fragment was excised from pBps010 (3) and cloned into Eco RI digested pcDNA3.1 V5-His (4) to generate the DNA vaccine pBps110 (5). An Xba I digestion dropped out the *Burkholderia pseudomallei* gene sequence and left the translation initiation sequence (6). Re-ligation of the DNA generated the plasmid pcDNA 3.1 Start (7).

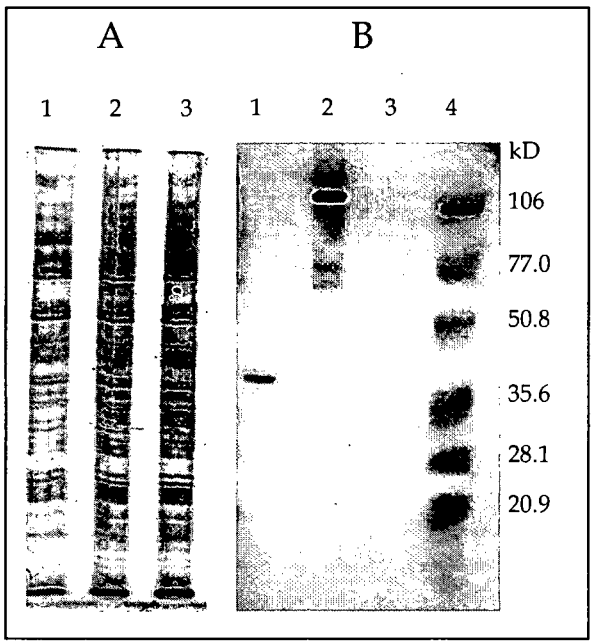

Figure 3: In vitro expression of the antigen encoded in the DNA vaccine.
Plasmid DNA was transfected into 293S cells using a cationic lipid complex. Cell extracts were prepared and separated by electrophoresis through a 12.5% poly-acrylamide gel, and either stained with coomassie blue (A) or transferred to a nitrocellulose membrane and probed with an anti-V5 epitope antibody (B). Lane 1, protein extracts from cells transfected with pBps110 (B. pseudomallei antigen 1); lane 2, pcDNA3.1 Lac Z; or lane 3 untransfected 293S cells. Lane 4 contains molecular weight standards indicated in kilo-Daltons (kDa).

DISTRIBUTION LIST

Generation of a Gene Expression Cassette to Enable the Rapid Production of DNA Vaccines

David Proll and Penelope Gauci

AUSTRALIA

DEFENCE ORGANISATION

	No. of copies
Task Sponsor	
DGDHS	1
S&T Program	
Chief Defence Scientist	shared copy
FAS Science Policy	
AS Science Corporate Management	
Director General Science Policy Development	
Counsellor Defence Science, London	Doc Data Sheet
Counsellor Defence Science, Washington	Doc Data Sheet
Scientific Adviser Joint	1
Navy Scientific AdviserDoc	Data Sht & Dist List only
Scientific Adviser - Army	1
Air Force Scientific Adviser	Data Sht & Dist List only
Scientific Adviser to the DMO M&A	Data Sht & Dist List only
Scientific Adviser to the DMO ELL	Data Sht & Dist List only
CBRN Defence Steering Committee	
Chair: Colonel McKaskill DGLD	1
Platforms Sciences Laboratory	
Head CBRN DC:	1
Task Manager: Dr P. Gray	1
Authors: Dr D. Proll	1
P. Gauci	1
DSTO Library and Archives	
Library Maribyrnong	Doc Data Sheet
Library Edinburgh	1
Australian Archives	1
Capability Systems Division	
Director General Maritime Development	Doc Data Sheet
Director General Land Development	1
Director General Aerospace Development	Doc Data Sheet
Director General Information Capability Development	Doc Data Sheet
Office of the Chief Information Officer	
Deputy CIO	Doc Data Sheet
Director General Information Policy and Plans	Doc Data Sheet

AS Information Strategies and Futures — Doc Data Sheet
AS Information Architecture and Management — Doc Data Sheet
Director General Australian Defence Simulation Office — Doc Data Sheet

Strategy Group
Director General Military Strategy — Doc Data Sheet
Director General Preparedness — Doc Data Sheet

HQAST
SO (Science) (ASJIC) — Doc Data Sheet

Navy
SO (SCIENCE), COMAUSNAVSURFGRP, NSW — Doc Data Sht & Dist List
Director General Navy Capability, Performance and Plans, Navy Headquarters — Doc Data Sheet
Director General Navy Strategic Policy and Futures, Navy Headquarters — Doc Data Sheet

Army
ABCA National Standardisation Officer, Land Warfare Development Sector, Puckapunyal — e-mailed Doc Data Sheet
SO (Science), Deployable Joint Force Headquarters (DJFHQ) (L), Enoggera QLD — Doc Data Sheet
SO (Science) - Land Headquarters (LHQ), Victoria Barracks NSW — Doc Data & Exec Summ

Intelligence Program
DGSTA Defence Intelligence Organisation — 1
Manager, Information Centre, Defence Intelligence Organisation — 1 pdf

Defence Materiel Organisation
Head Aerospace Systems Division — Doc Data Sheet
Head Maritime Systems Division — Doc Data Sheet
Head Land Systems Division — Doc Data Sheet

Defence Libraries
Library Manager, DLS-Canberra — Doc Data Sheet
Library Manager, DLS - Sydney West — Doc Data Sheet

OTHER ORGANISATIONS
National Library of Australia — 1
NASA (Canberra) — 1

UNIVERSITIES AND COLLEGES
Australian Defence Force Academy Library — 1
Serials Section (M list), Deakin University Library, Geelong, VIC — 1
Hargrave Library, Monash University — Doc Data Sheet
Librarian, Flinders University — 1

OUTSIDE AUSTRALIA

INTERNATIONAL DEFENCE INFORMATION CENTRES	
US Defense Technical Information Center	2
UK Defence Research Information Centre	2
Canada Defence Scientific Information Service	1
NZ Defence Information Centre	1
ABSTRACTING AND INFORMATION ORGANISATIONS	
Library, Chemical Abstracts Reference Service	1
Materials Information, Cambridge Scientific Abstracts, US	1
Documents Librarian, The Center for Research Libraries, US	1
SPARES	8
Total number of copies	**35 printed and 1 PDF**

DEFENCE SCIENCE AND TECHNOLOGY ORGANISATION
DOCUMENT CONTROL DATA

1. PRIVACY MARKING/CAVEAT (OF DOCUMENT)

2. TITLE

Generation of a Gene Expression Cassette to Enable the Rapid Production of DNA Vaccines

3. SECURITY CLASSIFICATION (FOR UNCLASSIFIED REPORTS THAT ARE LIMITED RELEASE USE (L) NEXT TO DOCUMENT CLASSIFICATION)

Document	(U)
Title	(U)
Abstract	(U)

4. AUTHOR(S)

David Proll and Penelope J. Gauci

5. CORPORATE AUTHOR

Platforms Sciences Laboratory
506 Lorimer St
Fishermans Bend Victoria 3207 Australia

6a. DSTO NUMBER	6b. AR NUMBER	6c. TYPE OF REPORT	7. DOCUMENT DATE
DSTO-TR-1597	AR-013-137	Technical Report	July 2004

8. FILE NUMBER	9. TASK NUMBER	10. TASK SPONSOR	11. NO. OF PAGES	12. NO. OF REFERENCES
2003/52379	03/009	DGDHS	18	36

13. URL on the World Wide Web

http://www.dsto.defence.gov.au/corporate/reports/DSTO-TR-1597.pdf

14. RELEASE AUTHORITY

Chief, CBRN Defence Centre

15. SECONDARY RELEASE STATEMENT OF THIS DOCUMENT

Approved for public release

OVERSEAS ENQUIRIES OUTSIDE STATED LIMITATIONS SHOULD BE REFERRED THROUGH DOCUMENT EXCHANGE, PO BOX 1500, EDINBURGH, SA 5111

16. DELIBERATE ANNOUNCEMENT

No Limitations

17. CITATION IN OTHER DOCUMENTS Yes

18. DEFTEST DESCRIPTORS

DNA vaccines, Molecular Biology, Plasmids, Biological Weapons and Vectors, genetic engineering

19. ABSTRACT

Recombinant DNA techniques have been used to engineer a DNA vaccine from a gene derived from Burkholderia pseudomallei. This vaccine was further manipulated to generate a gene expression cassette that can be used to readily generate new DNA vaccines, utilising genetic material from any source. The DNA vaccine was transfected into mammalian cells, grown in vitro, in order to assess the ability of the DNA to provide the cell with the necessary information for it to synthesise the protein encoded within the vaccine. Western blot analysis of these cell extracts resulted in the detection of a protein with a molecular mass predicted for the protein encoded by the DNA vaccine. This evidence suggests that transient transfection of a mammalian cell with the engineered plasmid DNA construct, resulting in the translation of the encoded protein has been successful. This in vitro process effectively mimics that which occurs when the DNA plasmids are used to vaccinate higher mammals.